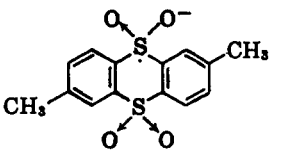
TABLE I

SUMMARY OF E.S.R. MEASUREMENTS[a]

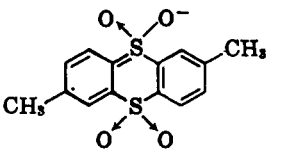
Anion-radical of compound	Color of radical	No. of major lines/splitting (gauss)[c]	Approx. no. fine lines	Total width of absorption, gauss
I	Blue	5/3.5	85	17.3
II	Blue	...	35[b]/70	14.5
III	Pale blue	5/2.1	5	8.9
IV	Pale blue	9/2.1	45	17.2

[a] Measurements were carried out at −30° on the potassium reduction products. The e.s.r. spectra are shown in Fig. 1. All radicals were stable indefinitely at the temperature of measurement. [b] Thirty-five lines of 0.41 gauss splitting were observed at 25°. [c] Peroxylamine disulfonate was the standard.

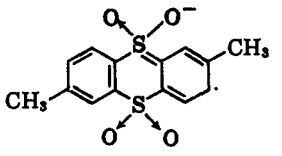
There is therefore no evidence for any interconversion of the different oxidation states of thianthrene under the conditions of our experiments. This behavior contrasts with that reported for thianthrene and its oxides in sulfuric acid. The positive ion-radicals of several of these compounds possess a common spectrum which has been interpreted as indicating that they have a common structure. *Cf.* J. M. Hirshon, D. M. Gardner and G. K. Fraenkel, *J. Am. Chem. Soc.*, **75**, 4115 (1953); J. E. Wertz and J. L. Vivo, *J. Chem. Phys.*, **23**, 2193 (1955); A. Fava, P. B. Sogo and M. Calvin, *J. Am. Chem. Soc.*, **79**, 1078 (1957); W. C. Needler, Ph.D. Thesis, University of Minnesota, 1961, University Microfilms, Inc.; H. J. Shine and L. Piette, *J. Am. Chem. Soc.*, **84**, 4798 (1962). The last two references cite evidence from the e.s.r. spectra of the positive ion-radicals of substituted thianthrenes which indicates that positions 2, 3, 7 and 8 of the thianthrene skeleton have the greatest spin density just as we have found for the anion-radicals of III and IV. We thank Professor Shine for the opportunity to read his manuscript prior to publication.



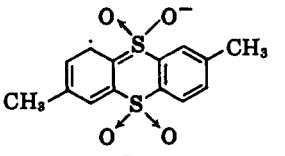
IVa



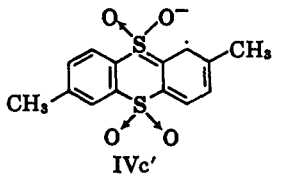
IVb



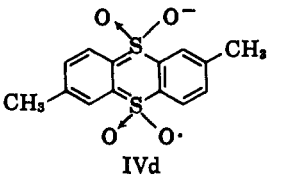
IVb'



IVc



IVc'



IVd

In conclusion, we have shown that sulfonyl groups can participate in the delocalization of odd electrons in aromatic systems. Furthermore, sulfonyl groups are more effective in this regard than sulfide groups.

Acknowledgment.—The authors wish to express their gratitude to Professors S. I. Weissman and D. Lipkin for many helpful discussions.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL, BOSTON 15, MASS.]

Proton Magnetic Resonance of Purine and Pyrimidine Derivatives. X. The Conformation of Puromycin

BY OLEG JARDETZKY

RECEIVED SEPTEMBER 25, 1962

An analysis of the 60 Mc. high resolution proton resonance spectrum of puromycin is presented. It is concluded that the conformation of the ribose ring in this compound corresponds to the previously discussed C'_3-endo form and that the entire molecule exists in a folded configuration.

1. Introduction

The antibiotic puromycin (6-dimethylamino-9-[3-deoxy-3-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]-β-purine) is thought to inhibit the assembly of the protein chain in the ribosomes by virtue of its structural similarity to the terminal 3′-aminoacyladenylate on the transfer RNA.[1] Since the process of protein synthesis is highly stereospecific and since it appears that different ribonucleosides and ribonucleotides possess different conformations, as well as considerable conformational rigidity,[2-6] it is to be expected that the conformation of puromycin will be of importance for the molecular mechanism of its action. Some inferences concerning this conformation on the basis of the high-resolution proton magnetic resonance (p.m.r.) spectra of puromycin have proved feasible.

2. Results and Discussion

The 60 Mc. p.m.r. spectrum of 0.1 *M* puromycin in D_2O, obtained with the Varian V-4300B-HR spectrometer, is shown in Fig. 1. The peak assignments are based on previous nucleotide and amino acid work[6-8] and on measurements of line intensities. (All shifts, measured as described before,[2-7] are in c.p.s. referred to benzene as an *external* standard.) The spectrum shows several remarkable features, when compared to the spectra of other structurally similar compounds. (1) The coupling constant between the protons on the first two ribose carbons, $J_{1'2'} = 2.5$ c.p.s., which is considerably smaller than generally found in purine derivatives.[2-7] (2) The C'_5 protons are shifted to higher fields by approximately 45 c.p.s. *and are non-equivalent*, in contrast to other ribosides, while C'_4H is shifted to lower fields by ~10 c.p.s. (Table I). (3) The peaks attributable to the aliphatic protons of phenylalanine are only slightly shifted to lower fields compared to their position in the free amino acid,[8] but the coupling constant between them is small and the peaks are broad (this feature is not entirely obvious from Fig. 1, but becomes clearly apparent when the HDO peak is shifted to lower fields by the addition of acid). (4) The aromatic peaks of phenylalanine are likewise broadened by comparison to either the free amino acid, or to other *p*-substituted phenyl derivatives at comparable concentrations in D_2O,[9,10] while the relaxation times of the adenine peaks are not changed. The magnitude

(1) M. B. Yarmolinsky and G. L. De la Haba, *Proc. Natl. Acad. Sci., U. S.* **45**, 1721 (1959).
(2) C. D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 229 (1960).
(3) C. D. Jardetzky, *ibid.*, **84**, 62 (1962).
(4) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).
(5) O. Jardetzky and C. D. Jardetzky, to be published.
(6) O. Jardetzky, to be published.
(7) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
(8) O. Jardetzky and C. D. Jardetzky, *J. Biol. Chem.*, **233**, 383 (1958).
(9) O. Jardetzky, *ibid.*, in press.
(10) N. Weiner, P. Pappas and O. Jardetzky, *Biochem. Pharmacol.*, **8**, 115 (1961).

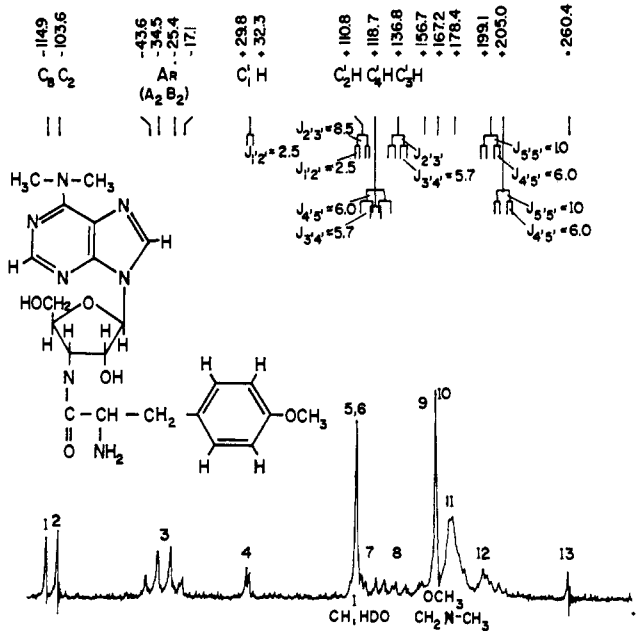


Fig. 1.—Sixty Mc. high resolution proton magnetic resonance spectrum of puromycin (0.1 M in D_2O, pH $\sim$ 3). Shifts are in c.p.s. from benzene used as an external reference standard, with the peaks assigned as: (1) C_8H of purine ring; (2) C_2H of purine ring; (3) aromatic protons of phenylalanine; (4) $C_1'H$ of ribose ring; (5) HDO solvent; (6) $C_2'H$ of ribose and CH of phenylalanine; (7) $C_4'H$; (8) $C_3'H$; (9) CH_2 of phenylalanine; (10) $-OCH_3$; (11) $H_3C-N-CH_3$; (12) $C_5'H$; (13) acetone used as an internal standard.

of the coupling constant, $J_{1'2'}$, suggests that the pentose conformation in puromycin is different from that found in most other known purine derivatives and the magnitudes of the other coupling constants support this view.

TABLE I

COMPARISON OF RIBOSE SHIFTS IN DIFFERENT ADENINE DERIVATIVES AT 60 MC.

	C'1H	C'2H	C'3H	C'4H	C'5H	C'5H
Adenosine	+37.0	+121.2	+130.1	+138.8	+154.4	
2'-AMP	+23.0	+ 90.1	+116.0	+137.8	+158.1	
3'-AMP	+32.5	+108.2	+101.5	+125.5	+154.8	
5'-AMP	+26.5	+120.3	+127.2	+132.0	+144.2	
Puromycin	+31.2	+110.8	+136.8	+118.7	+199.1	+205.0

For the present, the exact dihedral angles cannot be predicted from the magnitude of a single coupling constant, inasmuch as the relationship formulated by Karplus[11,12] was found not to be generally applicable.[12a] Nevertheless, certain conclusions can be drawn in special cases—for example, of ring systems for which *all* pertinent coupling constants are known, such as the case under discussion. Thus, if one merely assumes that the relationship between coupling constants J and dihedral angles φ is of the type $J = A + B\cos^2\varphi$, with maxima at 0 and 180° and a minimum at 90°,[12b] the *relative* magnitudes of the coupling constants observed in puromycin (*i.e.*, $J_{2'3'} > J_{4'5'} > J_{3'4'} > J_{1'2'}$) are found to be consistent with the 3'-endo ribose conformation found in certain pyrimidine riboside derivatives and shown in Fig. 2, but not consistent with the more usual 2'-endo conformation found in other purine derivatives,[2,3,5] as well as in the pyrimidine deoxyribosides (examined so far). In addition, they are inconsistent with a planar configuration, as discussed before.[2,3]

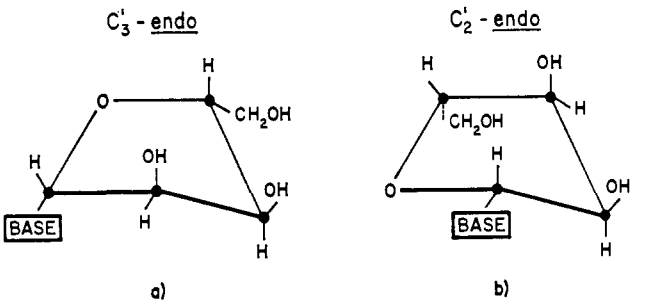


Fig. 2.—Bond orientations (a) in 3'-endo and (b) in 2'-endo ribose conformation.

The non-equivalence of the two C'_5 protons and the relative broadening of their resonance lines is of considerable interest, since it is not found in many other ribose derivatives. In the usual case the C'_5 lines are either a simple doublet (*e.g.*, Fig. 5, ref. 3), or part of an AB_2 system and are comparatively sharp. (Only a few direct relaxation measurements have been made on these compounds so far, but the values of T_2 fall into the range 0.5–1 sec., while they are of the order of 0.1 sec. as measured from the line width at half-height in the present case.) Both features strongly suggest that in puromycin the rotation about the C'_4–C'_5 bond of ribose is restricted, in contrast to most other ribose derivatives. This conclusion rests as much on the dissimilarity of the C'_4H–C'_5H_2 spectrum of puromycin to that of other ribosides, as on its similarity to the C_2H–C_3H_2 spectrum of chloramphenicol.[9] The arguments of supporting this interpretation in preference to the alternative that shifts arising from an asymetrically substituted neighbor[13] are not completely averaged have been discussed in considerable detail for the chloramphenicol case[9] and need not be repeated here, inasmuch as they apply without modification. It is worth noting, however, that in addition the broadening of the C'_5H_2 lines could not be explained simply by the larger molecular weight of puromycin, compared with, for example, adenosine, since the relaxation time (and hence the line width) of a freely rotating terminal group is not markedly affected by an increase in molecular weight.[14,15] This point is well illustrated by the sharp $-O-CH_3$ resonance in the present case.[16]

The marked shift of the C'_5H_2[17] resonance to higher fields accompanied by a smaller shift of the C'_4H resonance to lower fields, shown in Table I, probably indicates that the two groups are found in the dia- and

(11) (a) M. Karplus and D. H. Anderson, *J. Chem. Phys.*, **30**, 6 (1959); (b) M. Karplus, *ibid.*, **30**, 11 (1959).

(12) (a) R. U. Lemieux, J. D. Stevens and R. R. Fraser, *Can. J. Chem.*, **40**, 1955 (1962). (b) The weight of experimental evidence suggests that a relationship of this general type does apply, although the empirical values of the coefficients A and B have not been established with certainty. A consistent set of values can be obtained from the measurements on rigid molecules which have been made to date, but the question of their general applicability must be regarded as open until the spectra of a larger series of rigid compounds have been completely analyzed. This work will be reported in a separate communication.

(13) H. S. Gutowsky, C. H. Holm, A. Saika and G. A. Williams, *J. Am. Chem. Soc.*, **79**, 4596 (1957).

(14) O. Jardetzky and C. D. Jardetzky, "Introduction to Magnetic Resonance Spectroscopy," in D. Glick, Ed., "Methods of Biochemical Analysis," Vol. IX, 1962, pp. 235–410.

(15) O. Jardetzky, *Advan. Chem. Phys.*, in press.

(16) The broadening of the $N-CH_3$ groups occurs at low pH only and may be partially attributed to the exchange of the proton on the nitrogen and partially to a restriction of rotation of the dimethylammonium group. A similar explanation could be advanced for the broadening of the α-CH peak of phenylalanine, but not for the broadening of the remaining phenylalanine peaks, since this is not observed in the free amino acid.

(17) The alternative assignment of peak 12 to the β-CH_2 group of phenylalanine can be ruled out, not only on the basis of a comparison of the chemical shifts to those in the amino acid, but also by the absence of the corresponding α-CH doublet with a coupling constant of ~6.0 c.p.s. This assignment would also require that the broad singlet appearing under the HDO peaks be assigned to one of the ribose protons, which does not allow a self-consistent interpretation of the ribose spectrum.

paramagnetic regions of the phenyl ring, respectively.[18] The possibility that this is a non-specific shift, resulting from a change in the diamagnetic anisotropy of the solute, is excluded both by the fact that the shift appears relative to other ribose protons and by the fact that the bulk susceptibility correction for the puromycin solutions, as compared to other nucleoside and nucleotide solutions, is of the order of 0–5 c.p.s. This is seen from a comparison of the position of the acetone resonance (0.5% used as an internal standard) in a 0.1 *M* solution of puromycin at +260.4 c.p.s. to that in a 0.1 *M* solution of adenosine at +256.0 c.p.s. The fact that the shifts of the acetone and ribose resonances are relatively insensitive to changes in hydrogen ion concentration has been established previously.[7] Taken in conjunction with the short relaxation times of the phenylalanine moiety (<0.2 sec.), this suggests that the ribose and the phenylalanine rings remain in a relatively fixed orientation with respect to each other.

An examination of molecular models reveals that the number of possible conformations of the puromycin molecule which are consistent with the observed spectrum is quite limited. An assignment of a precise conformation for the entire molecule is of course not possible, but the C'_5-proton shift requires that the mutual orientation of the ribose and the phenyl ring be of the general type shown in Fig. 3. The possibility that part of this shift is due to a shielding by the purine ring cannot be excluded. However, attributing the *entire* shift to a displacement of the C'_5H_2 group into the diamagnetic region of the latter is not consistent with the restriction of rotation in either the ribose or the phenylalanine moiety. If, for example, the molecule would be maximally stretched out, the rotation of the phenyl group would be expected to be as unrestricted as it is in the case of the free amino acid. With the main rotation occurring about the C_2–C_3 bond of the alanine residue, one could expect: (1) a coupling constant for the $-CH-\beta-CH_2$ protons of the order of 6 c.p.s., (2) sharp resonance lines for the entire amino acid spectrum, and (3) additional shielding of the C'_2 and C'_3- protons of ribose, none of which are found. The more compact conformation shown in Fig. 3 (allowing for some variation in position) is also more consistent with the distortion of angles in the ribose as compared to those of the similarity substituted 3′-adenosine monophosphate (AMP), although a plausible alternative explanation is the larger size of the substituent group.

(18) C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).

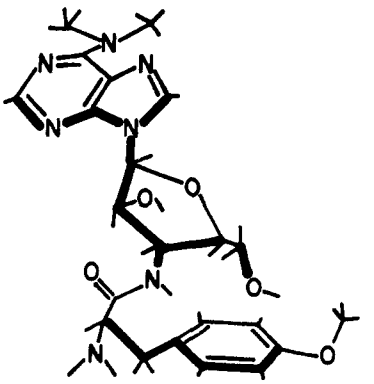


Fig. 3.—Puromycin in a folded conformation.

The reasons for this type of conformation in the case of puromycin are not immediately apparent. The C'_3-endo type of pucker is consistent with the notion that a bulky substituent on a carbon atom will tend to force this atom out of the plane of the ring, as appears to be the case for 2′-AMP. On the other hand, there is no obvious compelling reason for the folding of the molecule and a restriction of rotation in the phenylalanine side chain. The steric relationships are favorable to the formation of a hydrogen bond between the C'_5-hydroxyl and the π-electron system of the phenyl ring. The occurrence of such hydrogen bonds in aromatic solvents has been suggested, *e.g.*,[19] but the bonds are relatively weak (1–4 kcal./mole) and thus far there seems to be no evidence for their existence in strongly hydrogen-bonded solvents, such as water or D_2O. In contrast, hydrogen bonding to the methoxy group is not feasible, while hydrogen bonding to the carbonyl group would not account for the selective shielding of the C'_5 protons. It is conceivable that the molecule (which is positively charged in this *p*H range) is held in this configuration by reasonably stable water bridges, but additional evidence is clearly necessary to elucidate this point.

3. Experimental

The n.m.r. spectra were obtained on a Varian Associates V4300 B 60 Mc. high resolution spectrometer. The procedures were the same as described previously.[7,9] Puromycin was obtained as the dihydrochloride from the Lederle Division of the American Cyanamid Co. The D_2O (99.8% isotopic purity) was purchased from the Bio-Rad Corp., Richmond, Calif.

(19) A. W. Baker and A. T. Shulgin, *J. Am. Chem. Soc.*, **80**, 5358 (1958).

[CONTRIBUTION FROM THE PSYCHIATRIC RESEARCH UNIT, UNIVERSITY HOSPITAL, SASKATOON, SASKATCHEWAN, CAN., AND THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

Chemistry of Catecholamines: Revised Structures for the Iodoaminochromes

BY R. A. HEACOCK, O. HUTZINGER, B. D. SCOTT, J. W. DALY AND B. WITKOP

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The n.m.r. spectra of 22 indole compounds derived from the oxidation of noradrenaline, adrenaline, 3,4-dihydroxynorephedrine and isoproterenol have been determined. Their evaluation provided unambiguous proof for the position of the halogen in iodo- and bromoaminochromes. The halogen occupies position 7 and not 2 as presumed previously. Independent total syntheses of 5,6-dimethoxy-7-iodoindole (X) and of 5,6-dimethoxy-7-iodo-2-methylindole (XII) confirmed this assignment.

Introduction

Oxidation of adrenaline (I) with potassium iodate gives a deep red-violet solution,[1,2] from which in 1937 Richter and Blaschko[3] isolated deep red prisms of what they considered to be 3-hydroxy-2-iodo-1-methyl-2,3-dihydroindole-5,6-quinone (II, X = I).[3] In the same year Green and Richter[4] reported the isolation of an analogous

(1) S. Frankel and R. Allers, *Biochem. Z.*, **18**, 40 (1909).
(2) L. Kraus, *ibid.*, **22**, 131 (1909).
(3) D. Richter and H. Blaschko, *J. Chem. Soc.*, 601 (1937).
(4) D. E. Green and D. Richter, *Biochem. J.*, **31**, 596 (1937).